LentiFuge

Viral Concentration Reagent

User Manual

V2, 1/28/2015

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A. Introduction to LentiFuge

LentiFuge is a lentiviral purification reagent that allows for precipitation and concentration of virus without the use of high speed ultracentrifugation. The protocol for LentiFuge is only 2 hours long. The first hour is an incubation period with LentiFuge. The second hour is a standard centrifugation step in a Beckman JA-14, JA-10, or similar rotor. After these two short steps, the purified virus is simply resuspended and concentrated in the volume of choice and either used immediately or aliquoted and frozen. The amount provided (1 ml) will be sufficient to concentrate and purify virus from 1 liter of supernatant.

B. Required Equipment: Centrifuge, Rotors, and Centrifuge vessels

- Beckman Coulter Avanti J-E centrifuge (or similar)
    - JA-20 rotor with 30 ml capacity Nalgene polycarbonate centrifuge tubes (Fisher Scientific cat. # 3138-0050)
    - JA-14 rotor with 250 ml polycarbonate centrifuge bottles with Noryl sealing cap (Fisher Scientific cat. # 14-375-353)
- 0.2 µm polyethersulfone (PES) membrane filters

C. LentiFuge Protocol

1. Collect cell culture supernatant containing lentiviral vectors and pool in a sterile container. Filter supernatant through a 0.2 µm polyethersulfone (PES) membrane to remove packaging cells and cellular debris.
2. Transfer supernatant to a sterile centrifuge tube or bottle and add 1 µl of LentiFuge reagent per 1 ml of lentiviral supernatant.
3. Incubate supernatant containing LentiFuge for 1 hour at 4°C.
4. Centrifuge lentiviral supernatant/LentiFuge mixture at $\geq$12,000 RPM for at least 1 hour at 4°C under the following conditions:
    - For small-scale preparations (~30-35 ml supernatant from one 15 cm culture dish), we recommend the Beckman JA-20 rotor with 30 ml capacity Nalgene polycarbonate centrifuge tubes (Fisher Scientific cat. # 3138-0050).

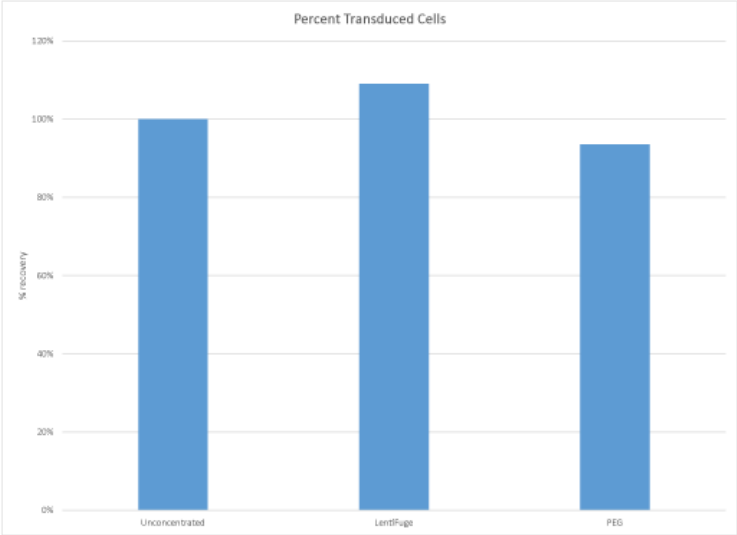
- For large-scale preparations of ~ 1 liter, we recommend the Beckman JA-14 rotor with four Fisherbrand 250 ml polycarbonate centrifuge bottles with Noryl sealing cap (Fisher Scientific cat. # 14-375-353).

Following centrifugation, the lentiviral particles may appear on the bottom or side of the tube as a faint white pellet. Pellets generated from small supernatant volumes may be difficult to see. It is advised to mark the centrifuge tube or bottle with a marker at the site where you expect the virus pellet to be.

5. Resuspend the pelleted lentiviral preparation in 1/100 of original volume using sterile phosphate buffered saline (PBS).
6. Aliquot the lentiviral suspension in sterile screw cap tubes and store at -80°C until ready to use.

D. Example Data

Lentifuge purified virus shows no titer loss and improved transduction efficiency compared to PEG-concentrated virus.



E. Technical Support

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F. Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of lentiviral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) are present in the packaged lentiviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Lentiviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous lentiviral sequences to form self-replicating virus or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

http://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with lentiviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

G. Terms and Conditions

Cellecta, Inc. Limited License

Cellecta grants the end user (the "Recipient") of the LentiFuge (the "Product") a non-transferable, non-exclusive license to use the reagents for internal research use only as described in the enclosed protocols; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Cellecta, Inc. -- separate licenses are available for non-research use or applications. The Product is not to be used for human diagnostics or included/used in any drug intended for human use. Care and attention should be exercised in handling the Product by following appropriate research laboratory practices.

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